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**ENVIRONMENT CANADA  
CONSERVATION AND PROTECTION  
ENVIRONMENTAL PROTECTION SERVICE  
PACIFIC AND YUKON REGION**

**TOXICITY TO RAINBOW TROUT  
OF SELECTED FORMULATIONS OF  
2,4-D, AND 2,4-D/ DIESEL OIL MIXTURES**

**Regional Program Report 85-02**

**By**

**M.T. Wan, R. Watts, and D. Moul**

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ABSTRACT

In 1983/1984 static fish bioassays were conducted by the Environmental Protection Service, Pacific Region, to compare the acute toxicity to rainbow trout of various formulations of 2,4-D and their mixture with different concentrations of diesel oil.

2,4-D dimethylamine (DMA) was the most toxic formulation to rainbow trout (96 hr LC<sub>50</sub> = 58.6 mg/L); followed by 2,4-D iso-octyl ester (IOE), (96 hr LC<sub>50</sub> = 70.3 mg/L); and 2,4-D diethanolamine (DEA), (96 hr LC<sub>50</sub> = 314.9 mg/L). The addition of 5% diesel oil to the 2,4-D formulations increased the toxicity to rainbow trout by 3.2 times for 2,4-D DEA, 1.7 times for 2,4-D DMA, and 2.6 times for 2,4-D IOE.

The implications of these results and their impact on fish are discussed.

### RÉSUMÉ

En 1983 et 1984 le Service de la protection de l'environnement de la région du Pacifique (Environnement Canada) a réalisé un programme de dosages biologiques statiques sur des poissons afin de déterminer et de comparer le degré de toxicité aiguë exercé sur les poissons par différentes formulations de 2,4-D et leur mélange avec différentes concentrations d'huile diesel.

La diméthylamine 2,4-D fut le composé le plus toxique pour la truite arc-en-ciel (96 hr  $LC_{50}$  = 58,6 mg/L); suivi par l'ester iso-octylique 2,4-D (96 hr  $LC_{50}$  = 70,3 mg/L) et la diéthanolamine (96 hr  $LC_{50}$  = 314,9 mg/L). L'addition de 5% d'huile diesel aux formulations de 2,4-D a augmenté la toxicité pour la truite arc-en-ciel par 2.3 fois pour la diéthanolamine, par 1.7 fois pour la diméthylamine et par 2.6 fois pour l'ester iso-octylique.

Le rapport examine les conséquences de ces résultats et leurs impacts sur les poissons.

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CONCLUSIONS

1. The toxicity of 2,4-D to rainbow trout varies with the type of formulation.
2. The addition of 5 percent diesel oil (by volume) to the herbicide/water mixtures increases the toxicity to rainbow trout of the DEA, the DMA, and IOE formulations of 2,4-D by 3.1, 1.6 and 2.6 times respectively.
3. Studies involving 2,4-D diesel oil mixtures should not be terminated prior to 120 hours of exposure to allow for the stabilization of LC50 values.
4. The toxicity to rainbow trout of 2,4-D IOE increases with increasing concentrations of diesel oil. No further increase in toxicity to rainbow trout results from increasing the oil concentration by greater than 25 percent.
5. The evaluation of herbicide test concentrations in water, presently based on calculations of volume/volume dilution (nominal value), should in future be verified by analyzing water samples from the test vessels in the fish bioassay studies.

## 1 INTRODUCTION

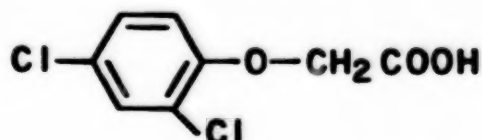
2,4-D is a post-emergent herbicide developed by AmChem Products Inc. in 1942. It is used for the selective control of broad-leaved vegetation in agriculture, forestry, and on rights-of-way. In the coastal forest areas of British Columbia, this herbicide is used in conifer release, logging road rights-of-way, and forest rehabilitation programmes for the control of broad-leaved plants, such as alder, maple, birch, and other species.

2,4-D is available in the acid, amine, and ester formulations. In British Columbia, the most commonly used formulations in forestry are the 2,4-D dimethylamine (DMA), 2,4-D diethanolamine (DEA), and 2,4-D iso-octyl ester (IOE). Sometimes these formulations are mixed with various concentrations (volume/volume) of diesel oil, particularly the IOE formulation, in order to enhance the adherence and absorption of the herbicide into the plant. Until the present investigation there was little data available on the toxicity to fish of different formulations of 2,4-D in mixtures of diesel oil. Typically 2,4-D and water (85-90%) are mixed with diesel oil (15-10%) in actual spray mixtures. However, there were concerns about the toxicity of such herbicide-oil mixtures to salmonid species of fish. At the request of the British Columbia Fisheries Branch, and to assist the regional office of the Environmental Protection Service in the evaluation of provincial permit applications for aerial and ground based use of 2,4-D in oil mixtures, static fish bioassays were conducted in 1983/1984 to determine the toxicity to fish of these herbicide/oil mixtures.

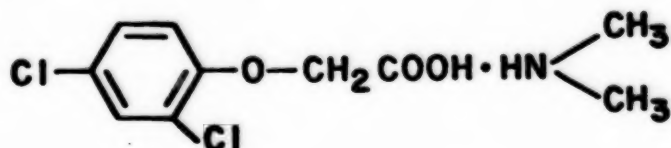
## 2 LITERATURE REVIEW

2,4-Dichlorophenoxyacetic acid (2,4-D) is the active ingredient in numerous chlorophenoxy herbicide products which are generally formulated as amine salts or esters of 2,4-D (Figure 1). Commercial products of 2,4-D commonly used in British Columbia include: 2,4-D DEA 500, Emulsamine E-3, Dacamine 4D, 2,4-D Amine 500, Estamine Brush Killer, Esteron 600, and Guardsman 2,4-D ester.

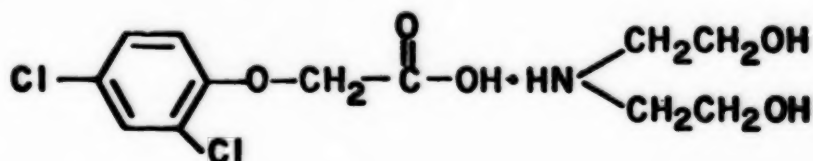
### 2,4-D (2,4-dichlorophenoxyacetic acid)



### Dimethylamine salt of 2,4-D



### Diethanolamine salt of 2,4-D



### Iso-octyl ester of 2,4-D

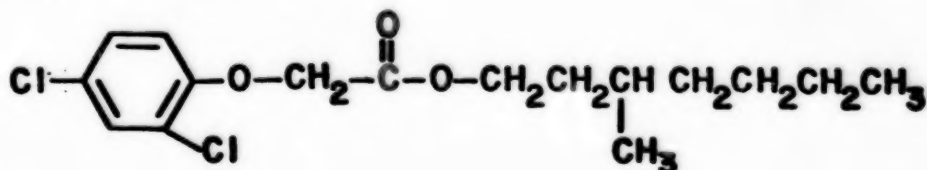


**FIGURE 1** STRUCTURAL FORMULAE OF 2,4-D AND ITS AMINE SALTS AND ESTERS

Previous acute static fish bioassays using different formulations of 2,4-D, conducted with bluegill (Pimentel, 1971; NRCC 1978; Lorz et al., 1979), indicated that the ester formulations of 2,4-D were generally more toxic (average 12 times more toxic) to fish than the amine formulations. Meehan et al. (1974) conducted a study to compare the toxicity of 2,4-D acid, 2,4-D butyl ester, and 2,4-D iso-octyl ester to several salmonid species. They demonstrated that 2,4-D butyl ester was by far the most toxic formulation to the salmonid species tested (Appendix I), and that juvenile pink salmon were exceptionally sensitive to all ester formulations of 2,4-D. At present there is limited information on the toxicity to salmonids of the various formulations of 2,4-D used in British Columbia coastal forest areas. Wan (1975) reported that 2,4-D amine (dimethyl/diethanolamine salts) was approximately 5 times less toxic to coho salmon than the ester (iso-octylester) counterpart. This study will determine the toxicity to rainbow trout of 2,4-D amine and 2,4-D ester formulations mixed with various amounts of diesel oil.

### 3 MATERIALS AND METHODS

#### 3.1 Bioassay Laboratory

The study was conducted at the Environmental Protection Service Aquatic Toxicity Laboratory, 1801 Welch Street, North Vancouver, British Columbia.

#### 3.2 Formulations Tested

The following formulations of 2,4-D and 2,4-D in mixtures of diesel oil were used for the fish bioassays:

- |                                |                                     |
|--------------------------------|-------------------------------------|
| A. 2,4-D Diethanolamine (DEA)  | G. 2,4-D IOE + 10% diesel oil       |
| B. 2,4-D DEA + 5% diesel oil   | H. 2,4-D IOE + 20% diesel oil       |
| C. 2,4-D Dimethylamine (DMA)   | I. 2,4-D IOE + 25% diesel oil       |
| D. 2,4-D DMA + 5% diesel oil   | J. 2,4-D IOE + 50% diesel oil       |
| E. 2,4-D Iso-octyl ester (IOE) | K. 2,4-D IOE + 75% diesel oil       |
| F. 2,4-D IOE + 5% diesel oil   | L. 100% diesel oil (pour point-9°C) |

Test mixtures of 2,4-D IOE in various proportions of diesel oil to water were prepared on a volume/volume basis in clean 500 ml volumetric flasks. These mixtures were well agitated by hand to ensure proper mixing prior to their use for toxicity testing. The required proportion of herbicide to oil to water for each of the test concentrations was measured out in clean graduated beakers.

#### 3.3 Test Fish

Underyearling rainbow trout (Salmo gairdneri) were obtained as fry from the B.C. Provincial hatcheries at Summerland (spring) and at Abbotsford (fall). Prior to testing, these fish were acclimated to laboratory conditions for a minimum period of one month. They were reared in fibreglass tanks at an ambient water temperature of 6°C (range, 4.5°C-8°C) and were fed a maintenance diet of Oregon moist pellets. Fish under test were not fed. The photo-period for holding, and during the tests was 14 hours of light (vita-lite) and 10 hours of darkness, with two 15 minute twilight periods.

### 3.4 Fish Culturing Conditions

Dechlorinated Vancouver City tap water was used for fish culturing. It also served as a diluent water for the tests. The average water quality values over a 4 month period were: temperature, 4.5°C-8°C; hardness, 10.2  $\pm$  5 mg/L CaCO<sub>3</sub>; conductivity, 10  $\pm$  5 umhos; pH, 6.1  $\pm$  1; alkalinity, 2.5  $\pm$  0.5 mg/L CaCO<sub>3</sub> (Appendix II).

### 3.5 Fish Testing Procedure

Fish bioassay procedures were based on protocols from Environment Canada (1980) and British Columbia Ministry of Environment (Buchanan, 1982). Glass aquaria (approximately 45 L capacity, measuring 27 x 21 x 54 cm) were used as the test vessels. A maximum volume of 30 litres of dechlorinated city tap water was used in each test vessel. Oil free compressed air was bubbled through the water column at a rate of 200 ml/min during the test and for a period of 30 minutes prior to the introduction of the herbicide/ oil mixtures. The required test mixture, prepared as described in Section 3.2, was poured into each test vessel and allowed to mix for 5-10 minutes to disperse the chemicals prior to the introduction of test fish. Ten fish were placed in each test vessel. Observations of fish mortality were recorded at 24, 48, 72, and 96 h after fish introduction. A fish was considered dead when all movement ceased and no further response could be obtained by prodding or moving the fish. Fish found dead during the test were removed at each observation. The results of cumulative fish mortality recorded after 48, 72 and 96 hours were used for the calculation of LC<sub>50</sub> values. These calculations were obtained by using a computer programme "Lethal" developed by Charles Stephans (6201 Coughdon Blvd. Duluth, Minn. 55804).

## 4 RESULTS AND DISCUSSION

### 4.1 Water Quality (diluent water) and Fish Conditions

The water quality of the diluent water for each of the test vessels was similar in all the bioassay tests, (Appendix II). Hardness as the calcium carbonate equivalent averaged 6.7 mg/L (range, 5.1-15.2), and the pH and water temperature remained constant at 6.2 and 15°C respectively. The test vessels were aerated at the rate of 200 ml per minute for 30 minutes. Meehan et al. (1974) emphasized that aeration of fish tank water is essential to prevent premature fish mortality, due in part to the depletion of dissolved oxygen. Fish loading density, length, and weight averaged 0.30 g/L, 4.5 cm, 0.77 g respectively, (Appendix II).

### 4.2 Toxicity to Rainbow Trout of Diesel Oil, 2,4-D and Mixtures of 2,4-D in Diesel Oil

The results of the fish bioassay tests (Table 1) indicated that diesel oil was the least toxic component to rainbow trout, (96 hour  $LC_{50}$  = 10,000 mg/L). However, when added to the herbicide preparation, diesel oil increased the toxicity of the final formulation, as indicated in Table 1.

For each of the 2,4-D formulations the  $LC_{50}$  values were determined at 48, 72, and 96 hours after the start of testing.  $LC_{50}$  values changed only slightly at 96 hours of exposure. Of the three 2,4-D formulations without the addition of diesel oil, 2,4-D DMA was the most toxic (96 h  $LC_{50}$  = 58.6 mg/L), followed by 2,4-D IOE (96 h  $LC_{50}$  = 70.3 mg/L), and 2,4-D DEA (96 h  $LC_{50}$  = 314.9 mg/L).

2,4-D DMA was slightly more toxic to Rainbow trout than 2,4-D IOE. This is in contrast to the results obtained with a similar test conducted in 1974, which demonstrated that 2,4-D DMA was 5 times less toxic to coho salmon than 2,4-D IOE (Wan, 1975). These two studies suggest that each fish species may respond differently to different formulations of 2,4-D.

When each of the 2,4-D formulations was tested with a 5% diesel oil component, the toxicity increased when compared to the  $LC_{50}$  values of the herbicide alone (Table 1). 2,4-D DEA with 5% diesel oil was 3.2 times more toxic; 2,4-D DMA with 5% diesel oil was 1.7 times more toxic; and 2,4-D IOE with 5% diesel oil was 2.6 times more toxic than each herbicide alone.

**TABLE 1** TOXICITY TO FISH (Rainbow trout) OF DIESEL OIL AND VARIOUS FORMULATIONS OF 2,4-D AND 2,4-D IN OIL MIXTURES\*

| TEST MATERIAL               | 48 h LC <sub>50</sub><br>(mg/L) | 72 h LC <sub>50</sub><br>(mg/L) | 96 h LC <sub>50</sub><br>(mg/L) |
|-----------------------------|---------------------------------|---------------------------------|---------------------------------|
| Diesel oil                  | > 27,167                        | > 27,167                        | 10,000                          |
| 2,4-D Diethanolamine (DEA)  | 350                             | 324                             | 314.9                           |
| 2,4-D DEA + 5% Diesel oil   | > 200                           | 133.2                           | 100.0                           |
| 2,4-D Dimethylamine (DMA)   | 65.7                            | 62.2                            | 58.6                            |
| 2,4-D DMA + 5% Diesel oil   | 38                              | 35.4                            | 35.4                            |
| 2,4-D Iso-octyl ester (IOE) | 79.9                            | 70.3                            | 70.3                            |
| 2,4-D IOE + 5% Diesel oil   | 26.8                            | 26.8                            | 26.8                            |
| 2,4-D IOE + 10% Diesel oil  | 40.4                            | 36.0                            | 28.0                            |
| 2,4-D IOE + 20% Diesel oil  | 15.6                            | 14.6                            | 10.1                            |
| 2,4-D IOE + 25% Diesel oil  | 9.0                             | 9.0                             | 7.9                             |
| 2,4-D IOE + 50% Diesel oil  | 7.0                             | 7.0                             | 4.2                             |
| 2,4-D IOE + 75% Diesel oil  | 13.8                            | 13.8                            | 9.7                             |

\*LC<sub>50</sub> values determined by non linear interpolation

Table 1 shows that 2,4-D IOE when mixed with increasing amounts of diesel oil generally produced increased toxicity to fish up to 50% concentration. The 96 h LC<sub>50</sub> value of 70.3 mg/L decreased to less than 10 mg/L when the proportion of diesel oil exceeded 20 percent (a 6.9 times increase in toxicity).

This study also demonstrated differences in the time required for the stabilization of LC<sub>50</sub> values. To minimize such discrepancies, it is suggested that future studies of herbicide/oil mixtures should be extended to 120 hours or longer, depending on the findings. In addition, this study indicated that of the two 2,4-D amines registered for use in forestry and other industries in British Columbia, 2,4-D DEA is the least toxic to fish and the more desirable formulation to use near fisheries sensitive areas.

The present study provides information on the toxicity to rainbow trout of 2,4-D amine and ester formulations. One more series of bioassay tests should be conducted exposing several salmonid species to the more common formulations of 2,4-D used in forestry in British Columbia, i.e., 2,4-D DEA, 2,4-D DMA, and 2,4-D IOE. The herbicide concentrations in this test series should also be verified by analyses of the water samples from the test vessels.

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APPENDICES

**APPENDIX I      TOXICITY TO JUVENILE SALMONIDS OF DIFFERENT FORMULATIONS OF 2,4-D\***

| SPECIES                   | 2,4-D FORMULATIONS (96 hr. LC <sub>50</sub> - mg/L) |             |                 |
|---------------------------|-----------------------------------------------------|-------------|-----------------|
|                           | Pure acid                                           | Butyl ester | Iso-octyl ester |
| Pink fry                  | 11.5                                                | < 1         | 1.5             |
| Chum fry                  | 36.8                                                | < 1         | 3.7             |
| Coho fry                  | 30.4                                                | < 1         | 17.9            |
| Sockeye smolts            | > 50                                                | < 1         | 7.4             |
| Coho fingerlings (Alaska) | > 50                                                | 2.2         | 63.3            |
| Dolly Varden fingerlings  | > 50                                                | 1.7         | 36.8            |
| Rainbow fingerlings       | > 50                                                | < 1         | 38.8            |
| Coho fingerlings (Oregon) | 33.3                                                | 1.5         | > 50            |

\*Adapted from: Meehan, W.R., L.A. Norris and H.S. Sears. "Toxicity of Various Formulations of 2,4-D to Salmonids in Southeast Alaska". J. Fisheries Board of Canada. 1974. 31(4), 480-485.

APPENDIX II DILUENT WATER QUALITY AND SIZE OF TEST FISH (rainbow trout)

| FORMULATIONS                       | pH  | TOTAL HARDNESS<br>(mg/L CaCO <sub>3</sub> ) | TEMPERATURE<br>(°C ± 1) | FISH LOADING<br>DENSITY (g/L) | FISH LENGTH<br>(cm) | FISH WEIGHT<br>(g)  |
|------------------------------------|-----|---------------------------------------------|-------------------------|-------------------------------|---------------------|---------------------|
| 2,4-D Diethanolamine               | 6.2 | 5.6                                         | 15                      | 0.25                          | 4.4<br>(3.5-5.1)    | 0.74<br>(0.40-1.00) |
| 2,4-D Dimethylamine                | 6.2 | 15.2                                        | 15                      | 0.20                          | 4.3<br>(3.6-5.1)    | 0.60<br>(0.40-1.00) |
| 2,4-D Dimethylamine<br>+ 5% diesel | 6.2 | 7.9                                         | 15                      | 0.19                          | 4.0<br>(3.2-4.8)    | 0.59<br>(0.30-1.10) |
| 2,4-D Iso-octyl ester<br>(IOE)     | 6.2 | 5.1                                         | 15                      | 0.40                          | 4.3<br>(3.5-5.0)    | 0.70<br>(0.30-1.00) |
| 2,4-D IOE + 5% diesel              | 6.2 | 5.1                                         | 15                      | 0.40                          | 4.3<br>(3.5-5.0)    | 0.70<br>(0.30-1.00) |
| 2,4-D IOE + 10% diesel             | 6.2 | 5.1                                         | 15                      | 0.50                          | 4.7<br>(4.0-5.4)    | 1.10<br>(0.60-1.40) |
| 2,4-D IOE + 20% diesel             | 6.2 | 5.1                                         | 15                      | 0.50                          | 4.7<br>(4.0-5.4)    | 1.10<br>(0.60-1.40) |
| 2,4-D IOE + 25% diesel             | 6.2 | 6.4                                         | 15                      | 0.20                          | 4.4<br>(3.9-4.8)    | 0.70<br>(0.40-0.90) |
| 2,4-D IOE + 50% diesel             | 6.2 | 6.4                                         | 15                      | 0.20                          | 4.5<br>(3.5-5.2)    | 0.70<br>(0.30-1.10) |
| 2,4-D IOE + 75% diesel             | 6.2 | 6.4                                         | 15                      | 0.20                          | 4.5<br>(3.5-5.2)    | 0.70<br>(0.30-1.10) |
| Diesel oil                         | 6.2 | 5.6                                         | 15                      | 0.28                          | 5.8<br>(3.5-5.1)    | 0.85<br>(0.50-1.25) |
| Average Values                     | 6.2 | 6.7<br>(5.1-15.2)                           | 15                      | 0.30<br>(0.19-0.50)           | 4.5<br>(4.0-5.8)    | 0.77<br>(0.59-1.10) |